

ON SEMPER'S METHOD OF MAKING DRIED PREPARATIONS.

BY DR. BENJAMIN SHARP.

Although this admirable method has been known and published for a number of years, it does not seem to have met with general acceptance. Many persons, indeed, with whom I have spoken do not seem to know of it at all, and for that reason I do not think it amiss to give an account of it here.

I have had the pleasure of working under Professor C. Semper, the discoverer of this method, for two years, and have seen, as well as prepared, many specimens. I have seen some specimens that have been prepared by this method over ten years ago, and not the slightest change has taken place in them, and they look as beautiful as those just finished.

The method requires close attention at certain stages, and the result depends upon the amount of care bestowed; the end, when successful, fully repays any amount of care that has been taken.

Nearly any animal or animal tissue may be prepared by this method; some require naturally more care than others—of fish, where there is a large quantity of fatty substance present, the greatest care is to be taken.

Dissections of animals are especially adapted for this method, and most of Prof. Semper's preparations are in this form. If desirable, when finished, the different systems of organs may be colored and thus serve as beautiful specimens for demonstration.

The object to be prepared is first placed in a solution of chromic acid of about $\frac{1}{4}$ to $\frac{1}{2}$ per cent., or even 1 per cent. In the case of dissections, these are to be prepared after the animal is killed and then placed in a dissecting tray, the bottom of which is filled with wax, so that different parts may be pinned out and thus better exposed to view; the tray may be then filled with the chromic acid solution.

The size and consistency of the object determines the length of time that it should remain in the solution; Annelides, small Gastropoda or Lamellibranchiata, small organs, as kidneys, etc., or small vertebrates, as frogs, mice, birds, etc., should remain in from six to eight hours; larger animals or organs from eight to twenty-four.

The chromic acid is merely to kill the tissues, and at the same

time hardens them somewhat. Any other of the hardening fluids may be used, and for these I can refer the reader to Dr. C. O. Whitman's paper on this subject, which appeared in the *American Naturalist*, (vol. xvi, 1882, pp. 697, 772). Chromic acid, however, is the reagent that Prof. Semper always uses, and it seems to answer every purpose.

After the object has been left a sufficient length of time in the fluid, this is poured off and the vessel filled with water, which should be constantly changed until there is no yellow color either in the object or in the water. In other words, as much of the acid must be withdrawn as possible. This part of the process is considerably shortened by allowing a current of water to flow through the vessel. This stage takes from ten to twenty hours, or even more.

After this is completed the object is placed in weak alcohol, from 30 to 40 per cent., for at least a day; when the specimen is quite small, ten or fifteen hours are sufficient. Then the alcohol may be strengthened to 60 or 70 per cent., and the object remain in this for two or three days (with larger objects, a week).

The object may now be placed in strong alcohol, from 90 to 95 per cent., for about the same length of time as with the 70 per cent. It may, indeed, remain here for weeks or months. I have often taken specimens that had been well preserved, after having been for a year in 90 per cent. alcohol, with as good a result as if freshly prepared.

In cases of dissections where parts have been pinned apart, after passing through the 70 per cent. alcohol stage, they may be taken carefully out of the trays, and the rest of the process gone through with in closely stopped bottles, for they are at this point quite stiff.

When objects have remained a sufficient length of time in the strong alcohol, they are placed in absolute alcohol. If the strong alcohol be changed once or twice, it will necessarily save the absolute alcohol to some extent.

This stage of absolute alcohol is the most critical part of the whole process. *Absolutely* every particle of the water must be removed, and the secret of the whole success depends on this one point. If any water be left in the tissue, it will become spotted and eventually spoil. I feel positive that those who have tried this method and have failed to produce satisfactory results, have

not been careful enough to remove every particle of water. I always take the precaution of changing the absolute alcohol once or twice, especially in moist climates.

After *all* the water has been withdrawn by the absolute alcohol, by remaining in it for three days to a week, the object is placed in turpentine, the best that can be procured. In this it is allowed to remain until it becomes thoroughly saturated—with large objects it is best to change the turpentine once. Two or three days are required for this stage. When saturated the object is quite stiff, and when the process is successful little or no contraction has taken place. The object is then placed in the air and protected carefully from the dust, and the turpentine allowed to evaporate. The object then soon presents a very beautiful appearance; it becomes white, resembling the whitest kid. It is light, stiff and, on account of the resin it contains, is perfectly insect-proof.

In annelides the iridescence is perfectly kept; hair and feathers retain their original colors.

If hollow organs, as the stomach, bladders, lungs, etc., are to be prepared, they may be blown up after they have been a short time in the turpentine; by so doing much space, and consequently much alcohol, are saved.

This is the practical part of the method, and I may add in a few words the whole principle. The object is to carefully and slowly harden the tissue and to *remove every particle of water*, the place of which is taken by the resin.

If the process be hurried contractions are apt to occur, and consequently bad-looking specimens result.

The *advantages* of this method are great. We have a perfectly dry object, with the perfect form kept; it is far preferable to handle than alcoholic dissections or preparations. It will last indefinitely and is insect-proof.

Prof. Semper keeps his preparations in glass boxes which are perfectly dust-proof, and by this both sides of the preparation can be distinctly seen.

An addition to this process was discovered by Prof. Semper about two years ago, which I do not think has yet been published. It is to place the prepared object in a solution of glycerine and sugar. In some objects this brings back almost entirely the original color of the animal; one disadvantage of this is, however,

that unless kept in dust-proof cases they would become spoiled by the dust collecting on them.

As absolute alcohol is so expensive in this country, the cost of a large specimen would be considerable, and therefore the process is better adapted for smaller objects.

A cheap method of making absolute alcohol, from the strong (95 per cent.) spirit, used in Prof. L. Rauvier's laboratory in Paris, would not, I think, be out of place to be mentioned here.

The details of this process were given me by my friend, Dr. W. Vignal, the assistant of Prof. Rauvier. A wide-mouthed bottle is taken, holding about a litre, and a three-quarters filled with the strong alcohol.

A mass of pulverized cupric sulphate ($\text{CuSO}_4 + 5 \text{ Aq.}$) is heated to a red heat in order to drive off the water of crystallization. This is poured, when cool, into the alcohol, the mouth of the bottle quickly closed, and the whole shaken. The cupric sulphate is insoluble in alcohol, but has an affinity for the water contained in it, and the water is consequently taken up, and the cupric sulphate becomes bluish. When this has stood—with occasional shakings—for a day or so, decant, and repeat the operation, especially if there is very much of a blue color in the sediment.

When finished a drop of alcohol can be mixed with a drop of turpentine on an object-glass, and if there be no particles of water to be seen under the microscope, the alcohol is absolute enough for all practical purposes.